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Image analysos of gut microflora.

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SUMMARY

In this thesis digital image processing is applied to the bacteriology of the human gut. Two motives underlie the work described here: gut flora is difficult to approach by classical bacteriologic means, and digital morphology may provide simple but rich descriptors of biologically relevant properties of the microbial ecosystem as a whole.

Chapter 1 is an introduction to gut bacteriology.

Motivated by the rich morphological variety observed in microscope slides made directly from suspensions of faecal bacteria, we decided to apply image processing to the problem. A necessarily brief overview of image processing, with emphasis on the techniques we used most, is given in Chapter 2.

A programming language, Med-Eyes, was implemented and is described in Chapter 3. This was necessary since at the time no commercial image analysis systems were available within budget which allow the easy implementation of the very specific algorithms needed for bacteriological analysis. Using this language as a tool, many potential descriptors of bacterial form were investigated. Finally, only area, perimeter, moment of inertia and convex hull area were left. Together, they accurately reflect size, oblong form and concavity of bacteria. By transforming to principal components this information was encoded into three uncorrelated new variables.

A survey and evaluation of staining and other experimental techniques employed is given in Chapter 4. Mainly for practical reasons, nigrosine staining and phase contrast microscopy were chosen for the remaining experiments. In this chapter it is demonstrated that variety of the fourth principal component is explained predominantly by measurement errors due to anisotropy in the image digitization process.

Chapter 5 describes theoretical and practical aspects of the characterization of pure cultures. It is shown that each of the species studied has its own morphometric distribution and that multinormal approximations to these distributions suffice to distinguish between species. Mixed data files were made, incorporating measurements from various species in known proportions. It turned out that one can analyse such files using the method of maximum likelihood, if the species used are not too much alike.

In Chapter 6 the gut flora of healthy untreated volunteers is studied. Seven parameters are introduced which describe the micromorphology of a sample of bacteria. They are means and medians of the three principal components, and an estimation of the entropy of their distribution (see Appendix A). A study is made of variations of these parameters in time and among subjects.

The first application of the method to individuals treated with antibiotics is described in Chapter 7. The parameters selected prove very sensitive to the disturbance introduced by treating human volunteers with ceftriaxone.

In summary, this thesis introduces a new method for morphometrical analysis of gut microflora. The most important single result from this work is the introduction of morphometrical entropy, which measures morphological diversity in a theoretically sound and practically useful way.